

## Original Article

# MicroRNA-148a-3p reduces inflammatory response by inhibiting IRS-1 and LDLR in diabetic foot ulceration

Junfang Yi<sup>1,2</sup>, Wenjiang Pan<sup>2</sup>, Xiaoqiang Li<sup>1,3</sup>

<sup>1</sup>Department of Vascular Surgery, The Second Affiliated Hospital of Soochow University, Suzhou 215000, Jiangsu Province, China; <sup>2</sup>Department of Vascular Surgery, Jinjiang Hospital, Jinjiang 362200, Fujian Province, China; <sup>3</sup>Department of Vascular Surgery, The Affiliated Drum Tower Hospital, Nanjing University Medical School, Nanjing 210008, Jiangsu Province, China

Received September 2, 2020; Accepted November 11, 2020; Epub January 15, 2021; Published January 30, 2021

**Abstract:** Objective: This study aimed to investigate the serum expression levels of MicroRNA-148a-3p, IRS-1 and LDLR in patients with diabetic foot (DF) and their relationship with inflammatory response. Methods: Forty-two patients with DF admitted to our hospital were enrolled as the study group. 43 diabetic patients without DF were enrolled as the disease control group, and 58 healthy individuals were selected as the healthy control group. The levels of MicroRNA-148a-3p, IRS-1 and LDLR expression in the serum of all participants were detected, and correlation analysis was performed between the serum levels and the clinical parameters, grades of severity for DF and inflammatory response. Results: The expression levels of MicroRNA-148a-3p, IRS-1 and LDLR in the study group, disease control group and healthy control group were statistically significant ( $P < 0.05$ ). The serum levels of MicroRNA-148a-3p were lower, and IRS-1 and LDLR were higher in patients with DF who developed inflammatory response than those in patients without DF ( $P < 0.05$ ). As the DF worsened, the serum level of MicroRNA-148a-3p gradually decreased, and the levels of IRS-1 and LDLR gradually increased ( $P < 0.05$ ). Conclusion: MicroRNA-148a-3p showed low expression in the serum of patients with DF who developed inflammatory reactions, and it was clinically possible to inhibit IRS-1 and LDLR by increasing the expression level of MicroRNA-148a-3p, so as to reduce inflammatory reactions in patients with DF.

**Keywords:** Diabetic foot, MicroRNA-148a-3p, IRS-1, LDLR

## Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia, which is caused by defective insulin secretion or other impaired biological function [1]. With the improvement of people's living standard and the change of dietary structure, the incidence rate of diabetes is rising year by year, reaching 6.4% worldwide. By 2035, the number of people with diabetes worldwide is expected to exceed 380 million [2, 3]. DM, commonly known as diabetes, is a metabolic disease that causes high blood glucose [4]. The hormone insulin promotes transfer of sugar from the blood into cells for storage or use as energy. With diabetes, your body either doesn't produce enough insulin or can't effectively use the insulin. Untreated high blood glucose caused by diabe-

tes can damage your nerves, eyes, kidneys, and other organs.

Diabetic foot (DF) is any pathology directly caused by peripheral arterial disease (PAD) and/or sensory neuropathy affecting the feet in DM. It is a long-term (or "chronic") complication of DM. Long-term high blood glucose level will cause damage to vascular endothelial cells, increase the degree of atherosclerosis, reduce vascular blood flow, and induce ischemic hypoxia in the lower limbs, leading to the formation of DF. According to the statistics by the International Diabetes Federation in 2015, 9.1-26.1 million diabetic patients worldwide develop foot ulcers each year [5]. Patients with DF ulcers have a 2.5 times higher risk of death within 5 years than diabetic patients without DF [6]. Diabetic patients exhibit low autoimmu-

nity. Gram-positive bacteria, negative bacteria, fungi, and even anaerobic bacteria that appear deep in the wound infection can trigger the inflammatory response in patients with DF. When patients with DF have inflammatory reactions in wounds, high blood glucose not only provides a good environment for bacteria growth, but also reduces the phagocytic capacity of white blood cells, making the wound difficult to heal. Antibiotics have been used to treat inflammatory reactions in patients with DF. However, the infection is mostly caused by mixed bacteria. Single antibiotic cannot achieve a good therapeutic effect, and over time, the strain tends to develop drug resistance or even mutation, reducing its efficacy. As the condition worsens, patients may experience discomfort, decreased quality of life, increased likelihood of hospitalization and amputation, and even death [7]. At present, domestic and foreign scholars aim to reduce the amputation rate of DF patients through early diagnosis and multi-disciplinary team management of complications, timely removal of callus and control of infection [8, 9].

The aim of this study was to investigate the relationship between MicroRNA-148a-3p, IRS-1 and LDLR and the wound inflammatory response, so as to provide a theoretical basis for reducing the wound inflammatory response in patients with DF.

### Materials and methods

#### General information

Forty-two patients with DF diagnosed by the endocrinology department in our hospital from January 2018 to December 2019 were enrolled as the study group, including 8 cases of grade 2, 14 cases of grade 3, 12 cases of grade 4, and 8 cases of grade 5. Forty-three diabetic patients without DF during the same period were selected as the disease control group. In addition, 58 cases of healthy individuals were recruited as the healthy control group. The baseline data of the three groups of patients in terms of gender, age, BMI index, etc. showed no statistically significant difference ( $P>0.05$ ).

Inclusion criteria: patients who met the diagnostic criteria for DF were classified to grades 2, 3, 4 and 5 according to classification standards of Wagner for diabetes [10].

Exclusion criteria included patients presenting with autoimmune system diseases, malignant tumors, severe cardiovascular diseases, severe psychiatric diseases, and other systemic infectious diseases [11].

A personal profile was established for the three groups of subjects, and information such as name, gender, age, contact telephone number, and residential address were registered, and an informed consent form was signed by each subject. This study was approved by the ethics committee of our institution.

#### Methods

*Determination of LDLR levels:* Peripheral venous blood was collected from the three groups of subjects for laboratory testing. EDTA was added as an anticoagulant and was mixed with the blood samples at room temperature for 15-20 min, followed by centrifugation at 1800 r/min at 2-8°C for 20 min. The supernatant was placed in the refrigerator at -80°C. If precipitates appeared in the storage process, centrifugation was performed again.

*Determination of LDLR levels using ELISA:* LDLR levels were determined according to the instructions of the LDLR ELISA kit (96-well plate, Shanghai Shuang Sheng Biotechnology Co.).

*Determination of IRS-1 levels:* Peripheral venous blood was collected from the three groups of subjects. Heparin as anticoagulant was added and centrifuged at 1000 r/min for 20 min at room temperature. The supernatant was stored in the refrigerator at -80°C.

The plasma levels of IRS-1 were determined using ELISA. The IRS-1 level was determined according to the instructions of Human Insulin Receptor Substrate 1 (IRS1) ELISA kit (96-well plate, Shanghai Jinma Experimental Equipment Co.).

*Determination of MicroRNA-148a-3p level:* Peripheral venous blood samples were collected, and real-time PCR was used to determine the level of MicroRNA-148a-3p [12].

(i) The content of total RNA in plasma was measured according to the Trizol LS kit (Invitrogen, USA) instructions, and the purity of RNA was determined by measuring absorbance with a

## MicroRNA-148a-3p reduces inflammatory response

**Table 1.** Comparison of baseline data in the three groups ( $\bar{x} \pm sd$ )/[n (%)]

Baseline data		Study group (n=42)	Disease control group (n=43)	Healthy control group (n=58)
Gender	Male	25	28	35
	Female	17	15	23
Average age (years)		55.8±6.71	55.2±6.04	55.7±5.33
BMI (kg/m <sup>2</sup> )		21.8±3.35	23.0±3.67	23.6±3.57
Average duration of diabetes (years)		8.0±1.52	5.3±2.15	

UV spectrophotometer. Absorbance = A260/A280.

(ii) The RNA was reverse transcribed into cDNA by reverse transcription kit (WD3126, Beijing Hua Yue Yang Biotechnology Co., Ltd.).

(iii) The expression level of MicroRNA-148a-3p was determined with cDNA as the template and U6 as the internal reference according to the instructions of the fluorescent quantitative PCR kit (BL705A, Biosharp).

### Observation indicators

**Comparison of MicroRNA-148a-3p expression levels in the three groups:** MicroRNA-148a-3p was associated with the regulation of diabetes, lipid metabolism and inflammation. According to the expression level of MicroRNA-148a-3p in cells of the three groups of subjects, the level of lipid metabolism and the inflammation can be determined. The high expression of MicroRNA-148a-3p indicates good regulation of lipid metabolism and the inflammatory response in patients with DF [13-16].

**Comparison of expression levels of IRS-1 in the three groups:** IRS-1 is a major substrate for the insulin receptor and other tyrosine kinases. It plays a key role in eliciting many actions of insulin, including the binding and activation of phosphatidylinositol (PI) 3-kinase and the subsequent increase in glucose transport. When IRS-1 expression is reduced, the insulin signaling is attenuated [17, 18]. IRS-1 is also a docking protein involved in angiogenesis, and when IRS-1 is inhibited, the inflammatory response is also suppressed, thereby alleviating the inflammatory response in wounds of patients with DF.

**Comparison of LDLR levels in the three groups:** LDLR is a transmembrane glycoprotein synthesized in the liver to regulate cholesterol metabolism in the body. The organism's inflammatory

response can cause damage to feet of patients with DF through negative feedback regulation of lipid metabolism. When LDLR expression is downregulated, the inflammatory response of wounds in patients with DF is alleviated [19-21].

### Statistical analysis

Statistical analysis was performed using SPSS 23.0. The measurement data were expressed in the form of mean  $\pm$  standard deviation ( $\bar{x} \pm sd$ ), and the t-test was used to compare the differences between groups.  $P < 0.05$  indicates significant difference.

## Results

### Comparison of baseline data in the three groups

The three groups of subjects were comparable in terms of baseline data such as sex, age, BMI, and course of disease ( $P > 0.05$ ) (**Table 1**).

### Comparison of serum MicroRNA-148a-3p, IRS-1, and LDLR levels in the three groups

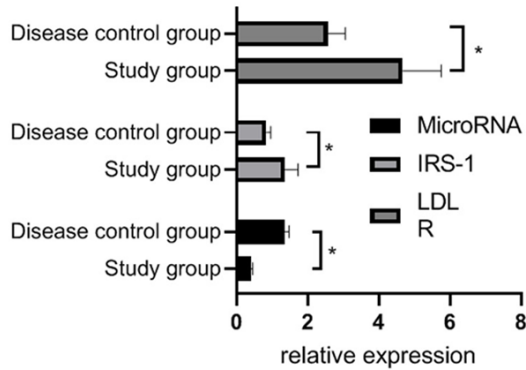
The study group showed significantly lower levels of MicroRNA-148a-3p, and higher levels of IRS-1 and LDLR than the disease control group ( $P < 0.05$ ) (**Figure 1**).

The disease control group showed lower serum levels of MicroRNA-148a-3p and higher levels of IRS-1 and LDLR than the healthy control group ( $P < 0.05$ ) (**Figure 2**).

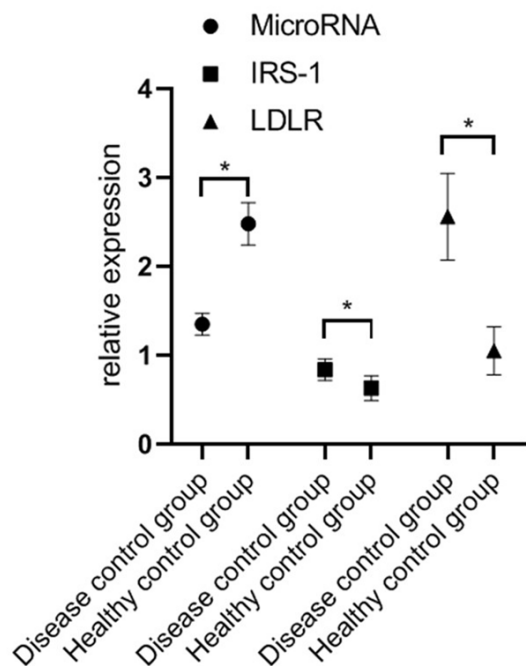
### Comparison of serum MicroRNA-148a-3p, IRS-1 and LDLR levels with regard to inflammatory response

The serum levels of MicroRNA-148a-3p in patients with DF and inflammatory response were lower while the levels of IRS-1 and LDLR

## MicroRNA-148a-3p reduces inflammatory response

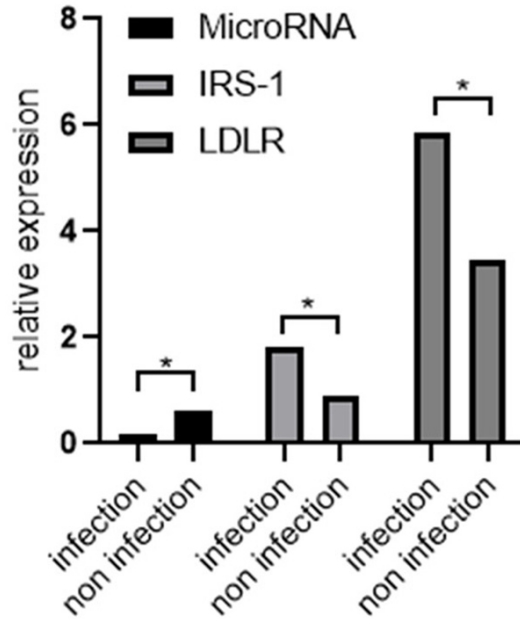


**Figure 1.** The expression levels of MicroRNA-148a-3p, IRS-1 and LDLR in the serum of subjects. The expression level of MicroRNA-148a-3p in serum of subjects in study group was significantly lower than that of subjects in disease control group ( $P<0.05$ ), and the expression levels of IRS-1 and LDLR of subjects in study group were significantly higher than those in disease control group ( $P<0.05$ ). \* Representing significant difference between the two groups.



**Figure 2.** MicroRNA-148a-3p, IRS-1 and LDLR expression levels. The expression level of MicroRNA-148a-3p in serum of subjects in disease control group was significantly lower than that of subjects in healthy control group ( $P<0.05$ ), and the expression levels of IRS-1 and LDLR of subjects in disease control group were significantly higher than those in healthy control group ( $P<0.05$ ). \* Representing significant difference between the two groups.

were higher than those in patients without inflammatory response ( $P<0.05$ ) (Figure 3).



**Figure 3.** Expression levels of MicroRNA-148a-3p, IRS-1 and LDLR in the serum of subjects with and without an inflammatory response in group A. Patients with DF and inflammatory response exhibited lower serum levels of MicroRNA-148a-3p and higher levels of IRS-1 and LDLR than those without inflammatory response ( $P<0.05$ ). \* Representing significant difference between the two groups.

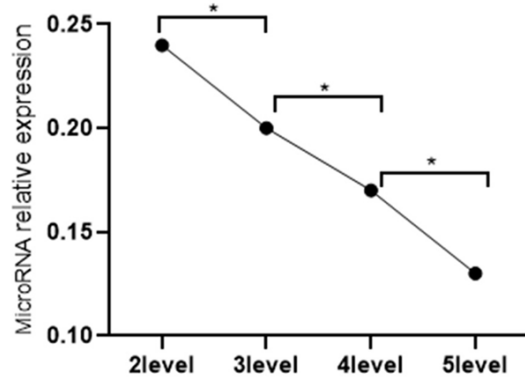
### Comparison of serum MicroRNA-148a-3p, IRS-1 and LDLR levels with regard to DF grading

The serum levels of MicroRNA-148a-3p, IRS-1 and LDLR among the patients with varying severity of DF were significantly different ( $P<0.05$ ). With the increase of DF grading, MicroRNA-148a-3p levels gradually decreased while IRS-1 and LDLR levels gradually increased, and the differences in these indicators among the patients with different grading were statistically significant ( $P<0.05$ ) (Figures 4-6).

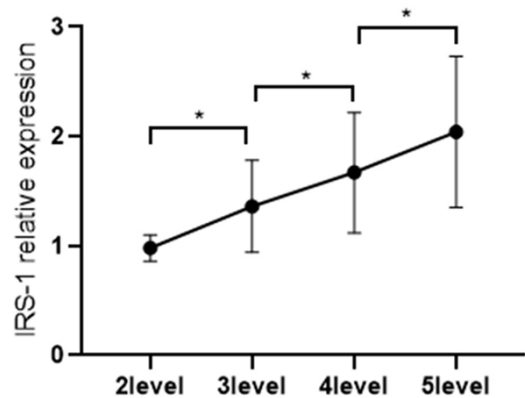
### Discussion

With the change in dietary habits, the incidence of diabetes continues to rise. It is incurable regardless of whether it belongs to type I or type II. The treatment options currently focus on controlling the patient's condition and preventing serious complications. DF is the most common complication, affecting about 30-40% of patients with diabetes [22, 23]. When patients develop DF, the risk of amputations and mortality rate as well as medical costs increase, which not only brings psychological

## MicroRNA-148a-3p reduces inflammatory response



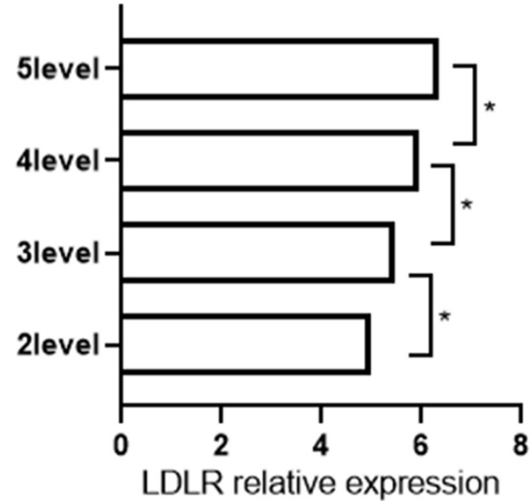
**Figure 4.** MicroRNA-148a-3p expression levels in the serum of subjects with different grading of diabetic foot. With the increase of DF grading, the expression levels of MicroRNA-148a-3p in serum of the subjects decreased significantly ( $P<0.05$ ). \* Representing significant difference between the two groups.



**Figure 5.** IRS-1 expression levels in patients with inflammatory reactions. With the increase of DF grading, the expression levels of IRS-1 in serum of the subjects increased significantly ( $P<0.05$ ). \* Representing significant difference between the two groups.

burden to patients and reduces the quality of life, but also brings serious economic burden to the family [24]. As the duration of the disease increases, the risk of DF increases. Therefore, timely intervention should be given in the early stages to reduce the incidence of DF.

MicroRNAs are highly conserved endogenous, single-stranded, non-coding RNAs with a length of 18-25 nucleotides, which are involved in physiological processes such as cell proliferation, differentiation, and apoptosis, and play a regulatory role in diabetes, tumors, cardiovascular and inflammatory responses [25]. Negative regulation of target gene expression is



**Figure 6.** LDLR expression levels in the serum of subjects with different grading of diabetic foot. With the increase of DF grading, the expression levels of LDLR in serum of the subjects increased significantly ( $P<0.05$ ). \* Representing significant difference between the two groups.

achieved by identifying the 3'UTR of the target gene through complementary base pairing [26]. The body usually promotes the release of anti-inflammatory factors when a wound appears, leading to automatic healing within 1-4 days. In patients with DF, the expression of inflammatory factors was increased, the duration of inflammatory was prolonged, angiogenesis was decreased, and an increased inflammatory response to wounds was observed in patients with DF [27, 28].

This study was conducted by measuring the expression levels of MicroRNA-148a-3p, IRS-1 and LDLR, and the results showed that the healthy control group had higher expression levels of MicroRNA-148a-3p and lower levels of IRS-1 and LDLR ( $P<0.05$ ) than the disease control and study groups. Patients with wound inflammation showed lower MicroRNA-148a-3p expression levels and higher IRS-1 and LDL expression levels than patients without wound inflammation in the study group. As the grade of DF patients increased, MicroRNA-148a-3p expression levels gradually decreased, and IRS-1 and LDLR expression levels gradually increased ( $P<0.05$ ). It was demonstrated that MicroRNA-148a-3p can significantly reduce the expression levels of IRS-1 and LDLR, thus inhibiting the wound inflammatory response for patients with DF.

## MicroRNA-148a-3p reduces inflammatory response

In the last two decades, microRNA targeted therapies have been developed rapidly, which can modulate multiple gene targets simultaneously and have become therapeutic options for cancer, diabetes, renal diseases, etc. The selection of targets was the key step for successful MicroRNA-targeted therapy. Several studies have confirmed that MicroRNAs have a regulatory role in wound inflammatory response, angiogenesis, and re-epithelialization [29-31]. MicroRNA-targeted drugs, e.g. Mir-cirasen, RG-101, RG-125/AZD4076, MRG-106, and MRX34, have entered the process of clinical trials for chronic diseases, but there is no known MicroRNA-targeted drug for DF at present.

In summary, MicroRNA-148a-3p regulates expression levels of IRS-1 and LDLR through negative feedback to reduce inflammatory responses in wounds of patients with DF. The novelty of this study is that it gave up the traditional anti-inflammatory regimen for DF and chose to study the serum expression levels of MicroRNA-148a-3p to explore the relationship between MicroRNA-148a-3p, IRS-1 and LDLR and the inflammatory response, so as to provide a theoretical basis for the development of MicroRNA-targeted drugs to inhibit the inflammatory response in patients with DF. The shortcomings of this study are as follows: (1) The sample size is small and geographically diverse, making the results less generalizable. (2) Only three groups were enrolled, and the results obtained may be biased. We will carry out in-depth studies with larger sample size to obtain more representative and scientific conclusions and provide more detailed theoretical basis for the treatment of DF.

### Disclosure of conflict of interest

None.

**Address correspondence to:** Xiaoqiang Li, Department of Vascular Surgery, The Affiliated Drum Tower Hospital, Nanjing University Medical School, No. 321 Zhongshan Road, Nanjing 210008, Jiangsu Province, China. Tel: +86-18906206898; E-mail: lixiaoqiang257@163.com

### References

[1] Petersmann A, Müller-Wieland D, Müller UA, Landgraf R, Nauck M, Freckmann G, Heine-

mann L and Schleicher E. Definition, classification and diagnosis of diabetes mellitus. *Exp Clin Endocrinol Diabetes* 2019; 127: S1-S7.

- [2] Guariguata L, Whiting DR, Hambleton I, Beagley J, Linnenkamp U and Shaw JE. Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Pract* 2014; 103: 137-149.
- [3] Zhang P, Lu J, Jing Y, Tang S, Zhu D and Bi Y. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis (†). *Ann Med* 2017; 49: 106-116.
- [4] American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2012; 35 Suppl 1: S64-71.
- [5] Armstrong DG, Boulton AJM and Bus SA. Diabetic foot ulcers and their recurrence. *N Engl J Med* 2017; 376: 2367-2375.
- [6] Walsh JW, Hoffstad OJ, Sullivan MO and Margolis DJ. Association of diabetic foot ulcer and death in a population-based cohort from the United Kingdom. *Diabet Med* 2016; 33: 1493-1498.
- [7] Singh R, Kishore L and Kaur N. Diabetic peripheral neuropathy: current perspective and future directions. *Pharmacol Res* 2014; 80: 21-35.
- [8] Frykberg RG and Banks J. Management of diabetic foot ulcers: a review. *Fed Pract* 2016; 33: 16-23.
- [9] Sinwar PD. The diabetic foot management - recent advance. *Int J Surg* 2015; 15: 27-30.
- [10] Izadi M, Kheirjou R, Mohammadpour R, Aliyoldashi MH, Moghadam SJ, Khorvash F, Jafari NJ, Shirvani S and Khalili N. Efficacy of comprehensive ozone therapy in diabetic foot ulcer healing. *Diabetes Metab Syndr* 2019; 13: 822-825.
- [11] Uchimura K, Ngamvithayapong-Yanai J, Kawatsu L, Ohkado A, Yoshiyama T, Shimouchi A, Ito K and Ishikawa N. Characteristics and treatment outcomes of tuberculosis cases by risk groups, Japan, 2007-2010. *Western Pac Surveill Response J* 2013; 4: 11-18.
- [12] Zeng J, Zhu L, Liu J, Zhu T, Xie Z, Sun X and Zhang H. Metformin protects against oxidative stress injury induced by ischemia/reperfusion via regulation of the lncRNA-H19/miR-148a-3p/Rock2 axis. *Oxid Med Cell Longev* 2019; 2019: 8768327.
- [13] Costantino S, Paneni F, Lüscher TF and Cosentino F. MicroRNA profiling unveils hyperglycaemic memory in the diabetic heart. *Eur Heart J* 2016; 37: 572-576.
- [14] Hathaway QA, Pinti MV, Durr AJ, Waris S, Shepherd DL and Hollander JM. Regulating microRNA expression: at the heart of diabetes mellitus and the mitochondrion. *Am J Physiol Heart Circ Physiol* 2018; 314: H293-H310.

## MicroRNA-148a-3p reduces inflammatory response

- [15] Saeedi Borujeni MJ, Esfandiary E, Taheripak G, Codoñer-Franch P, Alonso-Iglesias E and Mirzaei H. Molecular aspects of diabetes mellitus: resistin, microRNA, and exosome. *J Cell Biochem* 2018; 119: 1257-1272.
- [16] Wagschal A, Najafi-Shoushtari SH, Wang L, Godeke L, Sinha S, deLemos AS, Black JC, Ramírez CM, Li Y, Tewhey R, Hatoum I, Shah N, Lu Y, Kristo F, Psychogios N, Vrbanac V, Lu YC, Hla T, de Cabo R, Tsang JS, Schadt E, Sabeti PC, Kathiresan S, Cohen DE, Whetstine J, Chung RT, Fernández-Hernando C, Kaplan LM, Bernards A, Gerszten RE and Näär AM. Genome-wide identification of microRNAs regulating cholesterol and triglyceride homeostasis. *Nat Med* 2015; 21: 1290-1297.
- [17] Albegali AA, Shahzad M, Mahmood S and Ullah MI. Genetic association of insulin receptor substrate-1 (IRS-1, rs1801278) gene with insulin resistant of type 2 diabetes mellitus in a Pakistani population. *Mol Biol Rep* 2019; 46: 6065-6070.
- [18] Giandalia A, Pappalardo MA, Russo GT, Romeo EL, Alibrandi A, Di Bari F, Vita R, Cucinotta D and Benvenega S. Influence of peroxisome proliferator-activated receptor- $\gamma$  exon 2 and exon 6 and insulin receptor substrate (IRS)-1 Gly972Arg polymorphisms on insulin resistance and beta-cell function in southern mediterranean women with polycystic ovary syndrome. *J Clin Transl Endocrinol* 2018; 13: 1-8.
- [19] Jiang X, Yu J, Wang X, Ge J and Li N. Quercetin improves lipid metabolism via SCAP-SREBP2-LDLr signaling pathway in early stage diabetic nephropathy. *Diabetes Metab Syndr Obes* 2019; 12: 827-839.
- [20] Yu Q, Chen Y and Xu CB. Statins and new-onset diabetes mellitus: LDL receptor may provide a key link. *Front Pharmacol* 2017; 8: 372.
- [21] Zheng Y, Tang L, Huang W, Yan R, Ren F, Luo L and Zhang L. Anti-inflammatory effects of ang-(1-7) in ameliorating HFD-induced renal injury through LDLr-SREBP2-SCAP pathway. *PLoS One* 2015; 10: e0136187.
- [22] Guo Y, Song Z, Zhou M, Yang Y, Zhao Y, Liu B and Zhang X. Infiltrating macrophages in diabetic nephropathy promote podocytes apoptosis via TNF- $\alpha$ -ROS-p38MAPK pathway. *Oncotarget* 2017; 8: 53276-53287.
- [23] Wang X, Gao L, Lin H, Song J, Wang J, Yin Y, Zhao J, Xu X, Li Z and Li L. Mangiferin prevents diabetic nephropathy progression and protects podocyte function via autophagy in diabetic rat glomeruli. *Eur J Pharmacol* 2018; 824: 170-178.
- [24] Lazo-Porras M, Bernabe-Ortiz A, Sacksteder KA, Gilman RH, Malaga G, Armstrong DG and Miranda JJ. Implementation of foot thermometry plus mHealth to prevent diabetic foot ulcers: study protocol for a randomized controlled trial. *Trials* 2016; 17: 206.
- [25] Zhang Y, Qin W, Zhang L, Wu X, Du N, Hu Y, Li X, Shen N, Xiao D, Zhang H, Li Z, Zhang Y, Yang H, Gao F, Du Z, Xu C and Yang B. MicroRNA-26a prevents endothelial cell apoptosis by directly targeting TRPC6 in the setting of atherosclerosis. *Sci Rep* 2015; 5: 9401.
- [26] Neumann A, Napp LC, Kleeberger JA, Benecke N, Pfanne A, Haverich A, Thum T and Bara C. MicroRNA 628-5p as a novel biomarker for cardiac allograft vasculopathy. *Transplantation* 2017; 101: e26-e33.
- [27] Baltzis D, Eleftheriadou I and Veves A. Pathogenesis and treatment of impaired wound healing in diabetes mellitus: new insights. *Adv Ther* 2014; 31: 817-836.
- [28] Sorg H, Tilkorn DJ, Hager S, Hauser J and Mirastschijski U. Skin wound healing: an update on the current knowledge and concepts. *Eur Surg Res* 2017; 58: 81-94.
- [29] Li Q, Zhao H, Chen W, Huang P and Bi J. Human keratinocyte-derived microvesicle miRNA-21 promotes skin wound healing in diabetic rats through facilitating fibroblast function and angiogenesis. *Int J Biochem Cell Biol* 2019; 114: 105570.
- [30] Pizzino G, Irrera N, Galfo F, Pallio G, Mannino F, D'Amore A, Pellegrino E, Ieni A, Russo GT, Calapai M, Altavilla D, Squadrito F and Bitto A. Effects of the antagomiRs 15b and 200b on the altered healing pattern of diabetic mice. *Br J Pharmacol* 2018; 175: 644-655.
- [31] Umehara T, Mori R, Mace KA, Murase T, Abe Y, Yamamoto T and Ikematsu K. Identification of specific miRNAs in neutrophils of type 2 diabetic mice: overexpression of miRNA-129-2-3p accelerates diabetic wound healing. *Diabetes* 2019; 68: 617-630.